Acta Biochimica Polonica

Vol. 54 No. 1/2007, 199–204

on-line at: www.actabp.pl

Regular paper

A poly(ADP-ribose) synthetase inhibitor, benzamide protects smooth muscle cells but not endothelium against ischemia/reperfusion injury in isolated guinea-pig heart

Andrzej Jakubowski✉ and Magdalena Lomnicka

Department of Experimental Pharmacology, Chair of Pharmacology, Medical College of the Jagiellonian University, Kraków, Poland

Received: 12 April, 2006; revised: 26 February, 2007; accepted: 08 March, 2007
available on-line: 20 March, 2007

Activation of the nuclear enzyme poly(ADP-ribose) synthethase (PARS) is important in the cellular response to oxidative stress. During ischemia and reperfusion (I/R) increased free radical production leads to DNA breakage that stimulates PARS which in turn results in an energy-consuming metabolic cycle and initiation of the apoptotic process. Previous studies have reported that PARS inhibition confers protection in various models of I/R-induced cardiovascular damage. The purpose of this study was to determine the role of PARS inhibition in I/R-induced injury of smooth muscle cells and endothelium in the coronary circulation of the isolated guinea-pig heart. Control hearts and those treated with a PARS inhibitor — benzamide (100 μmol L^{-1}), were subjected to 30 min of subglobal ischemia and subsequent reperfusion (90 min). To analyze the functional integrity of smooth muscle cells and endothelium, one-minute intracoronary infusions of endothelium-independent (sodium nitroprusside, NaNP; 3 μmol L^{-1}) and endothelium-dependent (substance P, SP; 10 nmol L^{-1}) vasodilators were used before ischemia and at the reperfusion time. The degree of the injury of coronary smooth muscle and endothelial cells induced by I/R was estimated in terms of diminished vasodilator responses to NaNP (at 55 min and 85 min of reperfusion) and to SP (at 70 min of reperfusion), respectively, and expressed as the percentage of preischemic response. I/R reduced vasorelaxant responses to both vasodilators by half (to 54.1±5.1% and to 53.6 ± 4.9% of preischemic value for NaNP at 55 min and 85 min of reperfusion, respectively and to 45.9±6.5% for SP at 70 min of reperfusion). PARS inhibition provided complete restoration of vasorelaxation induced by NaNP (107.6±13.3% and 104±14.4% of preischemic response at the two time points of reperfusion, respectively). However, there was no effect on the SP-induced response (48+12.1% of preischemic response). We conclude that pharmacological PARS inhibition with benzamide protects coronary smooth muscle cells but not endothelium against I/R-induced reperfusion injury in the coronary circulation of the guinea-pig heart.

Keywords: PARS, I/R-induced reperfusion injury, coronary vessels, endothelium, smooth muscle cells

INTRODUCTION

Poly(ADP-ribose) synthetase (PARS) is one of the most abundant enzymes in the eukaryotic cell. Its main role is to maintain genomic integrity by aiding of damaged DNA. PARS catalyzes the cleavage of NAD^+ into nicotinamide and ADP-ribose and uses the latter for the synthesis of branched nucleic acid-like polymers — poly(ADP-ribose) (de Murcia *et al.*, 1994; Lindahl *et al.*, 1995). PARS activity play role in the regulation of cell replication and differentiation (Tanuma *et al.*, 1978; Bakondi *et al.*, 2002). Activated PARS utilizes cellular NAD^+ (Jacobson *et al.*, 1979) so the level of cellular ATP declines dra-

✉Author for correspondence: Andrzej Jakubowski, Department of Experimental Pharmacology, Chair of Pharmacology, The Jagiellonian University School of Medicine, Grzegórzecka Str. 16, 31-531 Kraków, Poland; e-mail: mfjakubo@cyf-kr.edu.pl

Abbreviations: Ach, acetylcholine; I/R, ischemia-reperfusion; NaNP, sodium nitroprusside; PARS, poly(ADP-ribose) synthetase; SP, substance P.

matically (Goodwin *et al.*, 1978; Sims *et al.*, 1983) and affects cell integrity. Moreover, low level of NAD^+ inhibits ATP regeneration and blocks reactivation of oxidized glutathione resulting in a lack of the main defensive mechanism against ischemic injury in the cells. All these changes lead to massive necrosis of cells subjected to ischemia/reperfusion (I/R). It has been shown that I/R stimulates generation of oxygen-derived free radicals (Lefer *et al.*, 1991), which can damage DNA and over-activate PARS. Therefore PARS inhibition at I/R should limit postischemic cell damage. In fact, many studies have shown beneficial effects of PARS inhibition in oxidative stress-related pathologies (Virag, 2005). It was proved that inhibition of PARS activity reduces heart damage in different models of ischemic cardiovascular injury (Thiemermann *et al.*, 1997; Farivar *et al.*, 2005; Nagata *et al.*, 2005). However, most *in vitro* and *in vivo* studies focused on post-reperfusion damage of myocardial function (Yamazaki *et al.*, 2004). Less is known about the role of PARS in the injury of vascular smooth muscle and endothelial cells (Mehta *et al.*, 1989; Lefer *et al.*, 1991). Current observations are not clear. Virag and Szabo (2002) showed that PARS inhibition with 3-aminobenzamide prevented the vasospasm caused by subarachnoid haemorrhage and improved vascular function in diabetes. Improved recovery of endothelial function was shown in pulmonary and splanchnic arteries in rats (Nagata *et al.*, 2005). On the other hand, Szabo group revealed in many studies that inhibition of PARS only partially protected vascular smooth muscle or endothelial cells exposed to various models of oxidative-related injury (Szabo *et al.*, 1996; 2004b; Andrasi *et al.*, 2005). Therefore the aim of the present study was to determine if PARS inhibition with benzamide reduces to the same degree I/R-induced injury of vascular smooth muscle cells and endothelium in the coronary circulation of the isolated guinea-pig heart.

MATERIALS AND METHODS

Chemicals. Acetylcholine (Ach), substance P (SP) and sodium nitroprusside (NaNP) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Benzamide was received as a kind gift from Professor Thiemermann (The William Harvey Research Institute, London, England).

Perfusion of the isolated guinea-pig heart. The method was described in details previously (Chlopicki & Gryglewski, 1993). Briefly, guinea-pig hearts were inversely perfused through the ascending aorta according to the Langendorff technique (Hugo Sachs Elektronik, HSE) with the Krebs–Henseleit buffer of the following composition (mmol L^{-1}): KCl 4.7, NaCl 118, $CaCl_2$ 2.52, $MgSO_4$ 1.64, $NaHCO_3$ 24.88, KH_2PO_4 1.18, glucose 5.55, sodium pyruvate 2.0, equilibrated with 95% O_2 + 5% CO_2 at 37°C. The heart was paced at 273 impulses per minute through platinum electrodes inserted to the right atrium. Left ventricular pressure (LVP) was measured using a fluid-filled balloon inserted into the left ventricle and connected to a pressure transducer (Isotec HSE). Heart systolic contractility was calculated from the LVP signal by an analogue differentiation amplifier (DIF module, HSE). Coronary flow was monitored by Narcomatic Electronic Flowmeter (HSE). All parameters were continuously displayed throughout the experiment and then analysed by a dedicated software (PSCF-IGEL, Poland). All experiments were completed in less than three hours.

Experimental protocol. Hearts were equilibrated at the perfusion pressure of 50 mm Hg for 10 min and then the pressure was raised to 60 mm Hg. The hearts were used for the experiment only if basal coronary flow was higher than 5 ml min^{-1} and bolus injection of acetylcholine (Ach, 300 pmoles) increased the flow by at least 3 ml min^{-1}.

Coronary vessels were tested for endothelium-dependent vasorelaxant responses with one-minute intracoronary infusions of substance P (SP, 10 nmol L^{-1}) and for endothelium-independent vasorelaxant responses with sodium nitroprusside (NaNP, 3 μmol L^{-1}).

Next, the hearts were subjected to 30 min of subglobal ischemia (90–95% reduction in coronary flow) followed by a 90 min reperfusion during which the vasodilator responses were tested again. NaNP was infused at 55 min and 85 min of reperfusion. SP as endothelium-dependent vasodilator was administered only once during reperfusion (at 70 min) because its repetitive administration (in time intervals shorter than 15 min) leads to a decrease in vasorelaxant responses (tachyphylaxis). The magnitude of all vascular responses was calculated as the area under the curve of coronary flow.

Control hearts were compared with hearts perfused with Krebs buffer containing benzamide (100 μmol L^{-1}) from the beginning of the experiment. The time schedule of I/R was established on the basis of previous experiments to obtain selective impairment of the response of coronary vessels to vasoactive substances with a relatively small effect on the basal coronary flow and heart contractility. In additional series of experiments the effect of benzamide (100 μmol L^{-1}) on basal hemodynamic functions and vasorelaxant responses was checked.

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and the experimental procedure used in the present study

Table 1. Effect of 30 min ischemia and 90 min of reperfusion (I/R) on coronary flow and heart systolic contractility of isolated guinea-pig heart.

Treatment with benzamide (100 μmol L^{-1}) did not affect listed parameters significantly (n=10).

Parameter	Time point					
	Basal	Treatment with benzamide (30 min)	I/R (45 min)	I/R (90 min)	I/R (45 min) + Benzamide	I/R (90 min) + Benzamide
Coronary Flow (ml min^{-1})	6.12±0.7	5.75±0.92	5.63±0.87	5.85±0.9	5.81±1.02	6.03±0.99
Heart systolic contractility (mm Hg s^{-1})	1021±93	949.5±95	969.9+91	991±85	1006±106	998±103

was approved by the Jagiellonian University Ethical Committee.

Statistics. Changes in vasodilator responses are shown as the percentage of basal preischemic values. Statistical significance between groups was evaluated by unpaired Student's *t*-test and within the group by paired *t*-test.

RESULTS

Basal coronary flow was 6.12±0.78 ml min^{-1} and heart systolic contractility was 1021±93 mm Hg s^{-1} (n=10).

Benzamide at a concentration of 100 μmol L^{-1} did not affect cardiac functions significantly. After a 30 min perfusion with benzamide, the basal coronary flow and heart systolic contractility were reduced by 6–7% (Table 1). Treatment with benzamide also did not change the vasodilator responses significantly. Vasodilatation caused by substance P (SP) was slightly higher (from 10% to 3% after 30 and 90 min, respectively) and by sodium nitroprusside (NaNP) smaller (from 10% to 4% after 30 and 90 min, respectively) in the presence of PARS inhibitor (Fig. 1).

Ischemia and subsequent reperfusion slightly, non-significantly, decreased coronary flow and heart systolic contractility. A thirty-minute ischemia and 45 min of reperfusion decreased coronary flow by 8% (ns) and heart systolic contractility by 5% (ns) as compared to basal values. A similar degree of impairment of coronary flow and heart systolic contractility was observed at the end of the reperfusion. In benzamide-treated hearts these values were almost identical (Table 1).

The effect of I/R on coronary vessel function was much more pronounced. I/R reduced endothelium-independent coronary responses to NaNP approximately by half, i.e. to 54.1±5.1% ($P<0.001$) and to 53.6 ± 4.9% ($P<0.001$) of preischemic values, at 55 min and 85 min of reperfusion, respectively. Similarly, the endothelium-dependent vasodilator re-

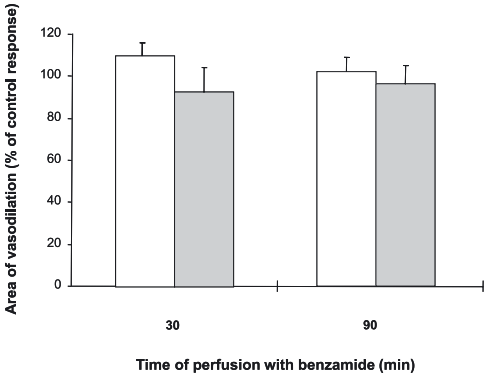

Figure 1. Effects of 90 min perfusion with benzamide (100 μmol L^{-1}) on coronary vessel responses to endothelium-dependent (substance P, SP) and endothelium-independent (sodium nitroprusside, NaNP) vasodilators in isolated perfused guinea-pig hearts.
Data expressed as percentage of control response. Open columns, vasodilatation evoked by SP (10 nmol L^{-1}); filled columns, vasodilatation evoked by NaNP (3 μmol L^{-1}), (n=10).

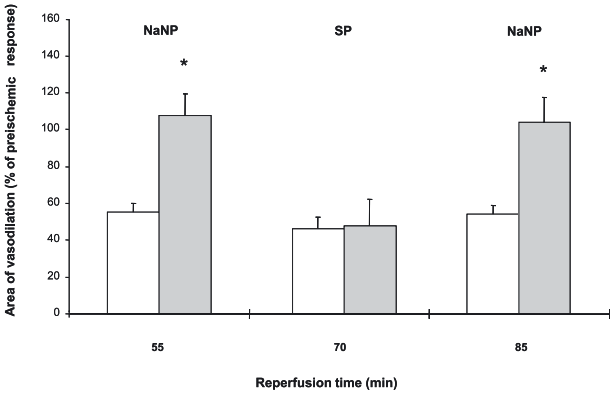

Figure 2. Effects of benzamide (100 μmol L^{-1}) on coronary vessel responses to endothelium-dependent (substance P, SP, 10 nmol L^{-1}) and endothelium-independent (sodium nitroprusside, NaNP, 3 μmol L^{-1}) vasodilators in isolated, perfused guinea-pig hearts subjected to 30 min subglobal ischemia and 90 min reperfusion.
Data expressed as percentage of preischemic response. Open columns, control hearts; filled columns, hearts treated with benzamide. (*$P<0.005$ between groups; n=10).

sponse to SP was depressed to 45.9±6.5% ($P<0.001$) at 70 min of reperfusion.

PARS inhibition protected the function of smooth muscle but not of the endothelial cells of coronary vessels against I/R-induced reperfusion injury. The suppression of endothelium-independent vascular responses to NaNP was totally reversed in benzamide-treated hearts. In this group, the increase in coronary flow reached 107.6±13.3% and 104.0±14.4% of preischemic values at 55 and 85 min of reperfusion, respectively ($P<0.005$ *vs* non-treated hearts). In contrast, the I/R-induced suppression of endothelium-dependent vasodilator responses to SP remained reduced to 48.0±12.1% of the preischemic value (ns *vs* non-treated group) (Fig. 2).

DISCUSSION

Although numerous studies describe the role of PARS activation in cell injury, only few investigated the effects of PARS activation in coronary circulation (Gilad *et al.*, 1997).

In the present study we demonstrated that benzamide, a well known PARS inhibitor which is three times more potent than the widely used 3-aminobenzamide, protected smooth muscle but not endothelial cells against I/R-induced injury of coronary vessels in the isolated guinea-pig heart. Inhibition of PARS activity did not affect significantly basal vascular tone or heart contractility. This is consistent with the PARS mode of action where at normal conditions the activity of this enzyme is rather low. However, it has been shown that DNA damage that occurs even after 15 min of ischemia and subsequent reperfusion increases PARS activity up to 500 times (D'Amours *et al.*, 1999; Virag & Szabo, 2002). This results in a dramatic increase in ATP consumption and impairment of mitochondrial respiration leading to severe cell damage. PARS activation in cultured endothelial cells in response to oxidative stress was described for the first time by Junod (Junod *et al.*, 1989). Additionally, it was shown that at the time of I/R smooth muscle and endothelial cells produce free radicals causing strong oxidative damage (Virag & Szabo, 2002). Our experiments showed that I/R led to significant impairment of coronary responses to both endothelium-dependent (SP) and endothelium-independent (NaNP) vasodilators. Because the over-activation of PARS has been shown in isolated heart models (Szabados *et al.*, 2000; Virag & Szabo, 2002), the use of benzamide should provide reasonable protection of the examined vascular bed. In fact, in our hands, benzamide totally reversed the damage of smooth muscle cells but failed to protect endothelial cells. Our observations are in accordance with the studies by Szabo (Szabo *et al.*, 2004a; 2004b) who showed that profound endothelial dysfunction was only partially reversed by PARS inhibition while vasodilator responses of smooth muscle cells were totally restored. On the other hand, many authors showed complete or at least significant protection also of endothelial cells by chronic or acute PARS inhibition in different vascular beds (Soriano *et al.*, 2001; Szabo *et al.*, 2002; Benko *et al.*, 2004).

This discrepancy shows that the effects of PARS inhibition are difficult to predict and strongly depend on the experimental model used, animal species as well as the inhibitor. Weaker or slower recovery of endothelial function after PARS inhibition (Szabo *et al.*, 1998b; 1998a) indicates that the coronary endothelium is more vulnerable to I/R-induced injury than other cells (Szabo *et al.*, 2002; 2004b; Pacher *et al.*, 2004). Indeed, Mizuno *et al.* (1997) demonstrated that after normothermic I/R-induced injury of pig heart, myocardial and endothelial function could be diversified: although the myocardial function showed a full recovery, the endothelial function remained impaired. Sack and coworkers (1997) showed, in human transplant biopsy specimens, that whereas myocyte integrity recovered within 60 min of reperfusion, regeneration of the endothelium lasted for up to a week.

Another possible explanation of the weaker protective effect of PARS inhibition in endothelial cells is only partial participation of PARS in the I/R-induced injury of these cells. Indeed, ATP level may not be the only one factor responsible for the impairment of endothelial-dependent response in reperfusion period. It was shown that PARS activation contributed to the expression of adhesive molecules like P-selectin and ICAM-1 in hearts subjected to global I/R, resulting in neutrophil activation and recruitment into the jeopardized tissue (Jerome *et al.*, 1993; Weyrich *et al.*, 1993; Gilad *et al.*, 1997), which is one of the crucial events for I/R injury. Inhibition of PARS activity in blood-perfused models may additionally prevent the critical neutrophil-endothelium interaction, which in turn leads to a stronger protection of these cells, while in our leukocyte-free model, this component of the beneficial effects was eliminated.

We did not study here the exact mechanisms of the beneficial role of PARS inhibition in the I/R-induced injury of the coronary vascular bed. However, the role of PARS over-activation in disruption of general cellular energetics is well established.

In summary, we showed that a PARS inhibitor — benzamide provided significant protection of smooth muscle but not of endothelial cells against I/R-induced injury in the coronary circulation of the isolated guinea-pig heart. Further studies are necessary to explain the mechanism of this phenomenon, and its relation to PARS inhibition.

REFERENCES

Andrasi TB, Blazovics A, Szabo G, Vahl CF, Hagl S (2005) Poly(ADP-ribose) polymerase inhibitor PJ-34 reduces mesenteric vascular injury induced by experimental cardiopulmonary bypass with cardiac arrest. *Am J Physiol Heart Circ Physiol* **288:** H2972–H2978.

Bakondi E, Bai P, Szabo EE, Hunyadi J, Gergely P, Szabo C, Virag L (2002) Detection of poly(ADP-ribose) polymerase activation in oxidatively stressed cells and tissues using biotinylated NAD substrate. *J Histochem Cytochem* **50:** 91–98.

Benko R, Pacher P, Vaslin A, Kollai M, Szabo C (2004) Restoration of the endothelial function in the aortic rings of apolipoprotein E deficient mice by pharmacological inhibition of the nuclear enzyme poly(ADP-ribose) polymerase. *Life Sci* **75:** 1255–1261.

Chlopicki S, Gryglewski RJ (1993) Nitric oxide is a major mediator in reactive hyperaemia evoked by a brief coronary occlusion in the guinea pig heart. *Eur J Pharmacol* **241:** 117–120.

D'Amours D, Desnoyers S, D'Silva I, Poirier GG (1999) Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. *Biochem J* **342** (Pt 2): 249–268.

de Murcia G, Schreiber V, Molinete M, Saulier B, Poch O, Masson M, Niedergang C, Menissier de MJ (1994) Structure and function of poly(ADP-ribose) polymerase. *Mol Cell Biochem* **138:** 15–24.

Farivar AS, McCourtie AS, Kinnon-Patterson BC, Woolley SM, Barnes AD, Chen M, Jagtap P, Szabo C, Salerno CT, Mulligan MS (2005) Poly (ADP) ribose polymerase inhibition improves rat cardiac allograft survival. *Ann Thorac Surg* **80:** 950–956.

Gilad E, Zingarelli B, Salzman AL, Szabo C (1997) Protection by inhibition of poly (ADP-ribose) synthetase against oxidant injury in cardiac myoblasts *in vitro*. *J Mol Cell Cardiol* **29:** 2585–2597.

Goodwin PM, Lewis PJ, Davies MI, Skidmore CJ, Shall S (1978) The effect of gamma radiation and neocarzinostatin on NAD and ATP levels in mouse leukaemia cells. *Biochim Biophys Acta* **543:** 576–582.

Jacobson EL, Lange RA, Jacobson MK (1979) Pyridine nucleotide synthesis in 3T3 cells. *J Cell Physiol* **99:** 417–425.

Jerome SN, Smith CW, Korthuis RJ (1993) CD18-dependent adherence reactions play an important role in the development of the no-reflow phenomenon. *Am J Physiol* **264:** H479–H483.

Junod AF, Jornot L, Petersen H (1989) Differential effects of hyperoxia and hydrogen peroxide on DNA damage, polyadenosine diphosphate-ribose polymerase activity, and nicotinamide adenine dinucleotide and adenosine triphosphate contents in cultured endothelial cells and fibroblasts. *J Cell Physiol* **140:** 177–185.

Lefer AM, Tsao PS, Lefer DJ, Ma XL (1991) Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. *FASEB J* **5:** 2029–2034.

Lindahl T, Satoh MS, Poirier GG, Klungland A (1995) Post-translational modification of poly(ADP-ribose) polymerase induced by DNA strand breaks. *Trends Biochem Sci* **20:** 405–411.

Mehta JL, Nichols WW, Donnelly WH, Lawson DL, Thompson L, terRiet M, Saldeen TG (1989) Protection by superoxide dismutase from myocardial dysfunction and attenuation of vasodilator reserve after coronary occlusion and reperfusion in dog. *Circ Res* **65:** 1283–1295.

Mizuno A, Baretti R, Buckberg GD, Young HH, Vinten-Johansen J, Ma XL, Ignarro LJ (1997) Endothelial stunning and myocyte recovery after reperfusion of jeopardized muscle: a role of L-arginine blood cardioplegia. *J Thorac Cardiovasc Surg* **113:** 379–389.

Nagata H, Horiguchi T, Enzan K, Nishikawa T, Suzuki K (2005) Inhibition of poly (ADP-ribose) synthetase improves pulmonary arterial endothelium-dependent relaxation after ischemic-reperfusion injury of splanchnic artery in rats. *Anesth Analg* **101:** 1799–1804.

Pacher P, Vaslin A, Benko R, Mabley JG, Liaudet L, Hasko G, Marton A, Batkai S, Kollai M, Szabo C (2004) A new, potent poly(ADP-ribose) polymerase inhibitor improves cardiac and vascular dysfunction associated with advanced aging. *J Pharmacol Exp Ther* **311:** 485–491.

Sack FU, Lange R, Mehmanesh H, Amman K, Schnabel P, Zimmermann R, Dengler T, Otto HF, Hagl S (1997) Transferral of extrathoracic donor neoplasm by the cardiac allograft. *J Heart Lung Transplant* **16:** 298–301.

Sims JL, Berger SJ, Berger NA (1983) Poly(ADP-ribose) polymerase inhibitors preserve nicotinamide adenine dinucleotide and adenosine 5′-triphosphate pools in DNA-damaged cells: mechanism of stimulation of unscheduled DNA synthesis. *Biochemistry* **22:** 5188–5194.

Soriano FG, Pacher P, Mabley J, Liaudet L, Szabo C (2001) Rapid reversal of the diabetic endothelial dysfunction by pharmacological inhibition of poly(ADP-ribose) polymerase. *Circ Res* **89:** 684–691.

Szabados E, Literati-Nagy P, Farkas B, Sumegi B (2000) BGP-15, a nicotinic amidoxime derivate protecting heart from ischemia reperfusion injury through modulation of poly(ADP-ribose) polymerase. *Biochem Pharmacol* **59:** 937–945.

Szabo C, Zingarelli B, Salzman AL (1996) Role of poly-ADP ribosyltransferase activation in the vascular contractile and energetic failure elicited by exogenous and endogenous nitric oxide and peroxynitrite. *Circ Res* **78:** 1051–1063.

Szabo G, Bahrle S, Batkai S, Stumpf N, Dengler TJ, Zimmermann R, Vahl CF, Hagl S (1998a) L-Arginine: effect on reperfusion injury after heart transplantation. *World J Surg* **22:** 791–797.

Szabo G, Fazekas L, Bahrle S, MacDonald D, Stumpf N, Vahl CF, Hagl S (1998b) Endothelin-A and -B antagonists protect myocardial and endothelial function after ischemia/reperfusion in a rat heart transplantation model. *Cardiovasc Res* **39:** 683–690.

Szabo G, Bahrle S, Stumpf N, Sonnenberg K, Szabo EE, Pacher P, Csont T, Schulz R, Dengler TJ, Liaudet L, Jagtap PG, Southan GJ, Vahl CF, Hagl S, Szabo C (2002) Poly(ADP-Ribose) polymerase inhibition reduces reperfusion injury after heart transplantation. *Circ Res* **90:** 100–106.

Szabo G, Liaudet L, Hagl S, Szabo C (2004a) Poly(ADP-ribose) polymerase activation in the reperfused myocardium. *Cardiovasc Res* **61:** 471–480.

Szabo G, Soos P, Bahrle S, Zsengeller Z, Flechtenmacher C, Hagl S, Szabo C (2004b) Role of poly(ADP-ribose) polymerase activation in the pathogenesis of cardiopulmonary dysfunction in a canine model of cardiopulmonary bypass. *Eur J Cardiothorac Surg* **25:** 825–832.

Tanuma SI, Enomoto T, Yamada MA (1978) Changes in the level of poly ADP-ribosylation during a cell cycle. *Exp Cell Res* **117:** 421–430.

Thiemermann C, Bowes J, Myint FP, Vane JR (1997) Inhibition of the activity of poly(ADP ribose) synthetase reduces ischemia-reperfusion injury in the heart and skeletal muscle. *Proc Natl Acad Sci USA* **94:** 679–683.

Virag L (2005) Structure and function of poly(ADP-ribose) polymerase-1: role in oxidative stress-related pathologies. *Curr Vasc Pharmacol* **3:** 209–214.

Virag L, Szabo C (2002) The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacol Rev* **54:** 375–429.

Weyrich AS, Ma XY, Lefer DJ, Albertine KH, Lefer AM (1993) *In vivo* neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. *J Clin Invest* **91:** 2620–2629.

Yamazaki K, Miwa S, Ueda K, Tanaka S, Toyokuni S, Unimonh O, Nishimura K, Komeda M (2004) Prevention of myocardial reperfusion injury by poly(ADP-ribose) synthetase inhibitor, 3–aminobenzamide, in cardioplegic solution: in vitro study of isolated rat heart model. *Eur J Cardiothorac Surg* **26:** 270–275.